NASA TECHNICAL TRANSLATION NASA TT F-15,804

STUDIES ON THE DETECTION OF CLINICALLY SILENT INVOLVEMENT OF MUSCLE IN LUPUS ERYTHEMATOSUS

H. Nebe, Th. Thormann, E. Barthelmes, H. Klug, G. Weishaupt, E. Apostoloff and N. Sönnischen

Translation of "Untersuchungen zur Erfassbarkeit klinisch stummer Muskelbeteiligung beim Lupus erythematodes," Dermatologische Monatschrift, Vol. 160, No. 3, 1974, pp. 201-214

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 JULY 1974

STANDARD TITLE PAGE

1. Report No. NASA TT F-15,804	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle STUDIES ON THE DETECTION OF CLINICALLY SILENT INVOLVEMENT OF MUSCLE IN LUPUS ERYTHEMATOSUS		5. Report Date July 1974 6. Performing Organization Code
7. Author(s) H. Nebe, Th. Thormann, E. Barthelmes, H. Klug, G. Weishaupt, E. Apostoloff and N. Sönnischen, Dermatology Clinic and Polyclinic (Charity) and First Medical Clinic and Polyclinic (Charity), Humboldt University, Berlin		8. Performing Organization Report No. 10. Work Unit No. 11. Contract or Grant No.
9. Performing Organization Name and Address Leo Kanner Associates Redwood City, California 94063		13. Type of Report and Period Covered
12. Sponsoring Agency Name and Address National Aeronautics and Space Administration, Washington, D.C. 20546		14. Sponsoring Agency Code

15. Supplementary Notes

Translation of "Untersuchungen zur Erfassbarkeit klinisch stummer Muskelbeteiligung beim Lupus erythematodes," Dermatologische Monatschrift, Vol. 160, No. 3, 1974, pp. 201-214

16. Abstract Muscle biopsies from patients with different clinical pictures of lupus erythematosus with and without clinical symptoms of myopathy were tested by light microscopic, immunofluorescent microscopic and electron microscopic methods. Humoral muscular antibodies were detected by the antihuman globulin consumption test and the sarcolemma fluorescent antibody test. The histological methods proved to be the most reliable for determining lupus myopathy without clinical symptoms. No correlation was found between clinical and morphological lesions and the appearance of muscle antibodies in the serum of patients.

17. Key Words (Selected by Author(s))	18. Distribution Statement unlimited		
19. Security Classif. (of this report) Unclassified	20. Security Classif. (of this page) Unclassified	21. No. of Pages 24	22. Price 4.25

NASA-HQ

STUDIES ON THE DETECTION OF CLINICALLY SILENT INVOLVEMENT OF MUSCLE IN LUPUS ERYTHEMATOSUS

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Summary /201*

The detection of clinically silent and manifest involvement of muscle in different clinical types of lupus erythematosus (L.e.) was investigated with the aid of light microscopic, immunofluoresence microscopic, and electron microscopic examinations and the determination of humoral muscular antibodies by the antihuman globulin consumption test and the sarcolemma fluorescent antibody test.

Morphological examinations were confirmed as the most reliable diagnostic methods. The antihuman globulin consumption test has thus far proved to be unsuitable for our case pool; no specific conclusion can be drawn with regard to the sarcolemma fluorescent antibody test. There was no correlation between myopathic symptoms and paraclinical findings.

In L.e. chronic discoides localisatus, morphological changes could be detected, which were interpreted as partial manifestations of the immunological defense mechanism, and which were present to a pronounced degree in L.e. chronicus cum exacerbatione subacuta. In L.e. visceralis, characteristic immunoglobulin and complement deposits were found in the case of the focal myositis typical of L.e. These findings were also observed in a less pronounced form in L.e. chronicus discoides disseminatus.

* Numbers in the margin indicate pagination in the foreign text.

The humoral antibodies which were detected in L.e. visceralis independent of the clinical symptoms were viewed as immunological side effects to which no primary pathogenetic significance can be attributed.

* * *

In lupus erythematosus (L.e.), the musculature is believed to be affected just as frequently as joints and skin [4]. Like lupus arthritis and skin changes, the involvement of muscle can be an early symptom of L.e. [1]. However, it is frequently ignored when there are simultaneous arthritic complaints, or else the subjective symptoms are attributed to the poor general condition [4].

Since, in L.e., the musculature is massively attacked by inflammatory processes only in exceptional cases, a well-defined set of symptoms in the form of myalgia, myasthenia, musculature /202
swelling and myogelosis, paralysis, atrophy, myosclerosis and contracture can rarely be anticipated [5]. Most patients exhibit only mild forms of muscular involvement, which are clinically silent.

Observations of paraclinical changes, which pointed to visceral manifestations in patients with the clinical picture of lupus erythematosis chronicus discoides (L.e.chr.d.) [3, 13], impelled us to undertake studies to detect clinically silent involvement of muscle in L.e.chr. and L.e. chronicus cum exacerabatione subacuta (L.e.chr.c.ex.s.) in our own patient pool, and to compare the results with the findings for the myopathies present in L.e. visceralis (L.e.v.), in order to find a correlation between clinical symptoms and paraclinical changes, taking the different types of L.e. into account, and to discover the diagnostic value of the various examination methods.

Our Own Studies

Case Pool

Of the L.e. patients in ambulant care of the Dermatological Clinic of Charité, we selected those with L.e. chronicus discoides disseminatus (L.e.chr.d.d.), L.e. chronicus discoides localisatus (L.e.chr.d.l.), L.e.chr.c.ex.s., and L.e.v. with or without clinical symptoms of a lupus myopathy. The studies were conducted on 19 patients with L.e.chr.d.l., of which one patient complained of myositic symptoms, on three patients with L.e.chr.d.d. without clinical symptoms of lupus myopathy, on eight patients with L.e.chr.ex.s., of which two complained of muscular pain, and on four patients with L.e.v., of which three exhibited symptoms in the sense of muscular involvement.

Method

To detect morphological changes in the musculature, light microscopic, fluorescence microscopic, and electron microscopic studies were conducted on biopsies from the M. biceps brachii.

1. Light Microscopic Studies

The specimen preparations of skeletal musculature were divided up between the various methods of study. The portion assigned to histological examination was fixed in formalin and processed into paraffin sections (H.E. and van-Gieson staining, sometimes PAS reaction Hotchkiss-McManus).

2. Immunofluorescence Microscopic Studies (Direct Method)

A specimen from the M. biceps brachii biopsies was placed in the native state in liquid N_2 and sealed in an airtight

container, frozen for about 6 hours, and stored for a few days at -20°C.

The direct immunofluorescence histology (DIF) of Coons and Kaplan [2] was carried out in the modification of Gitlin et al. [6]. Unfixed cryostatic sections (4-5 nm) were air-dried for 20 min, and incubated for 30 min with the chromatographically purified (DEAE Sephadex A 50) IgG -- labelled with fluorescein isothiocyanate (FITC) -- of the following antisera (AS) in the given dilutions: antihuman IgG 1:10; antihuman IgA 1:2; antihuman IgM 1:2; antihuman β_{1C} 1:5; antihuman fibrinogen 1:10; antihuman albumin 1:2; and non-incubated control sections as well.

The working titers of the sera were comparatively low, because only monospecifically adsorbing conjugates were employed. The monospecificity was verified by means of Mancini's radial immunodiffusion, which is 10^2 times more sensitive than immunoelectrophoresis.

As controls, we conducted the inhibition test of Cherry et al. and examined healthy musculature. After imbedding in phosphate-buffered (pH 7.2) glycerin, we examined it under the Zeiss-Nf microscope with HBO 200 and "Tiyoda" dark-field capacitor; UG 1/1.5 and BG 12/2 exciter filters; GG 9/1 suppression filter.

3. Electron Microscope Studies /203

For the electron microscopy, the tissue specimens were cut up and fixed as usual in 1% buffered (pH 7.3) OsO_4 solution for 2 hours, and then imbedded in Vestopal W after dehydration. The contrast of the sections, which were between 500 and 800 Å thick, was enhanced with uranyl acetate and lead citrate, and examined with an acceleration voltage of 60 to 80 kV.

4. Steffen Antihuman Globulin Consumption Test (AGCT)

The antigen employed was homogenized and lyophilized muscle fibers from young persons obtained post amputationem from the M. quadriceps femoris.

The antihuman globulin consumption test of Steffen [15] was conducted as an elution technique in the modification of Miescher: 0.5 ml 1% muscle antigen suspension was incubated with 1 ml inactivated patient serum, washed seven times, and the antibodies then cleaved in 0.35 M NaCl solution at 60°C (20 min).

The test erythrocytes (OD) were covered with human anti-D serum (addition of AB serum) and then washed again.

For the test, 0.05 ml each of eluate and Coombs serum were mixed in the dilutions 1:32 to 1:2084, and then 0.05 ml of the covered erythrocytes was added. After 20 min, the reading was taken macroscopically by tipping the frosted-glass slides.

Controls: Each experiment was accompanied by the following controls: four negative comparison sera from healthy persons, one positive serum, the Coombs test without addition of an antibody up to 4096, the Coombs test with addition of NaCl solution without antigen incubation and of NaCl solution, which, in parallel with the experiment, was incubated with muscle antigen instead of with patient serum, and which was cleaved as above.

5. Immunofluorescence Microscopic Studies (Indirect Method) to Determine Humoral Sarcolemma Antibodies

The sera of patients with L.e.chr. were added in the native state to frozen-section preparations of the tissue material to be test (30 min at room temperature in moist chamber). Then the sections were washed three times. The presence of antigen-antibody

binding was detected in accordance with the Sandwich procedure of Weller and Coons [17] with the aid of an antihuman gamma globulin serum conjugated with fluorescein isothiocyanate (30 min at room temperature in moist chamber). After washing them three times, the preparations were imbedded in buffered glycerin solution. Cryostatic sections of rat diaphragm were employed as antigen carriers. Immediately after extraction, the tissues were frozen in liquid NN_2 [sic] and stored at -20°C. In the frozen-section technique, preparations about 10 µm thick were prepared which were treated as above. Direct-light microscopy was employed.

Results

Histomorphological examinations have proved to be the most reliable method for detecting clinically silent involvement of muscle in L.e., and there is a large measure of agreement between these results. There was no well-defined correlation with the clinical symptoms. The changes depicted in Figs. 1 and 2 were observed by light microscopy.

Since distinguishing normal histological findings or those within the "normal range of variation" from clearly inflammatory pathological changes can be very difficult and is often highly arbitrary, mild changes without any specific character, but which already point to a reaction in the sense of incipient myositis, were termed "mesenchymal activation." In circulatory disorders in the perimysium, the environmental picture showed slight polycytoses, consisting of histiocytes and lymphocytes, and mild edema in the endomysium. Degenerative alterations of the muscle fibers were completely absent. Such changes can already be interpreted as partial symptoms, manifested in the musculature, of general immunological processes. To a far higher degree, this is also true of the interstitial focal myositis with degenerative parenchymal changes of varying distinctiveness, which, as "lupus myopathy," shows largely characteristic features, but is not at

all specific. The localized distribution of the inflammation in /204
the muscle tissue is due to the special anatomy and innervation of skeletal muscle.

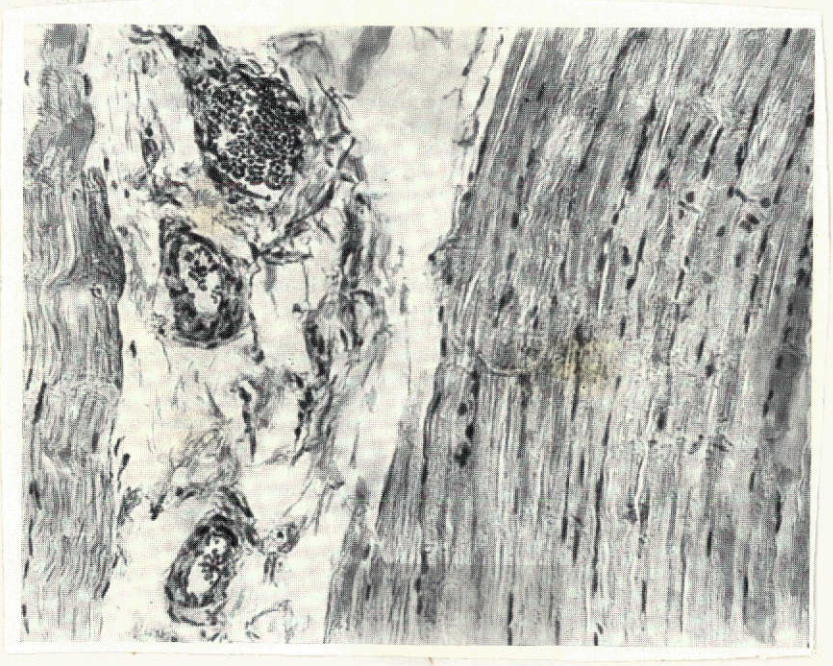

Fig. 1. Mesenchymal activation. HE. -- Magnification: 90 x. Enlarged, congested veins and mild edema of perimysium. Slightly increased salt content in endomysium. No degenerative changes detected in muscle fibers.

Light microscopic examinations were conducted on 19 patients /205
with L.e.chr.d.l. Eight of them exhibited muscle changes, which were detectable in five patients in the form of a mesenchymal activation and in two patients as rather indistinct focal myositis. None of these patients complained of subjective symptoms. No morphological changes were observed in patients exhibiting muscle symptoms.

In two of three patients with L.e.chr.d.d., one minor and one distinct focal myositis were detected, and there were also clinical manifestations in one female patient.

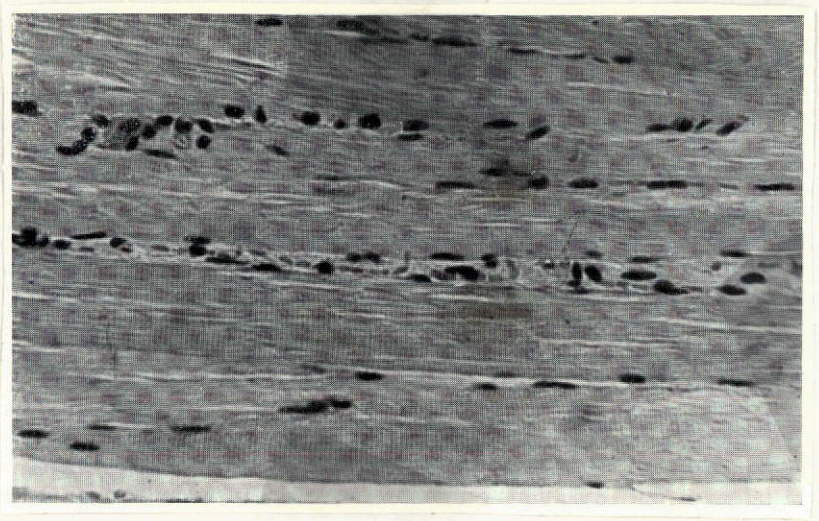

Fig. 2. Myositis. HE. -- Magnification: 230 x. Inflammatory infiltrates in endomysium. Degenerative changes of a muscle fiber with granular disintegration of sarcoplasm and invasion of lymphocytes and sarcolemma nuclei.

The studies conducted on seven patients with L.e.chr.c.ex.s. /206 found mesenchymal activation in three patients, which caused pain to one patient, and a weakly defined focal myositis without subjective symptoms in one patient.

In two women with L.e.v. and clinical signs of muscle involvement (myalgia and muscular atrophy), massive myositic changes were detected (Table 1).

The so-called vacuolization of the muscle fibers described in the literature and characterizing lupus myopathy was found in none of our preparations [8, 11, 12].

The method of direct immunofluorescence appeared to furnish the most reliable evidence, since localization and immunoglobulin class exhibit patterns characteristic for L.e.

A distinction was drawn between fluorescence of the sarcolemma and homogeneous or vascular fluorescence composed of individual

TABLE I. LIGHT MICROSCOPIC FINDINGS IN RELATION TO CLINICAL TYPES OF L.e. AND THE CLINICAL SYMPTOMS OF MUSCLE INVOLVEMENT

Patient	Clinical type of L.e.	Muscle symptoms	Light microscopic findings
R. E. ♂	L.e.chr.d.l.	Myasthenia, myalgia in lower extremities	ø
M. G. ♀	L.e.chr.d.l.	ø	Moderately distinct myositis
A. E. ♀	L.e.chr.d.l.	ø	ø
P. H. J. ♂	L.e.chr.d.l.	ø	ø
L. J. ♀	L.e.chr.d.l.	ø	ø
G. E. ♀	L.e.chr.d.l.	ø	Mesenchymal activation
G. E. ♀	L.e.chr.d.l.	ø	Mesenchymal activation
K. J. ♂	L.e.chr.d.l.	ø	Mesenchymal activation
L. J. ♀	L.e.chr.d.l.	ø	Minor focal myositis
P. H. ♀	L.e.chr.d.l.	Myalgia in lower extremities	ø
R. A. ♀	L.e.chr.d.l.	ø	ø
R. H. ♂	L.e.chr.d.l.	ø	ø
S. M. ♀	L.e.chr.d.l.	ø	ø
W. M. ♀	L.e.chr.d.l.	ø	ø
Z. I. ♀	L.e.chr.d.l.	ø	ø
K. Ch. ♀	L.e.chr.d.l.	ø	Mesenchymal activation, slight perimyositis
S. A. ♂	L.e.chr.d.l.	ø	ø
K. H. ♂	L.e.chr.d.l.	ø	Mesenchymal activation
Sch. H. ♂	L.e.chr.d.l.	ø	Mesenchymal activation
Sch. E. ♂	L.e.chr.d.d.	ø	ø
H. V. ♀	L.e.chr.d.d.	Myalgia in lower extremities	Slight myositis
N. H. ♂	L.e.chr.d.d.	ø	Chronic localized myositis
Sp. J. ♀	L.e.chr.c.ex.s.	ø	Moderately distinct myositis
V. L. ♀	L.e.chr.c.ex.s.	ø	ø
B. E. ♂	L.e.chr.c.ex.s.	ø	ø
M. J. ♀	L.e.chr.c.ex.s.	ø	ø
F. G. ♂	L.e.chr.c.ex.s.	ø	Mesenchymal activation
M. K. ♂	L.e.chr.c.ex.s.	ø	Mesenchymal activation
Z. K. H. ♂	L.e.chr.c.ex.s.	ø	Mesenchymal activation
H. Ch. ♀	L.e.v.	Myalgia, myasthenia of upper and lower extremities	Distinct focal myositis
Z. A. ♀	L.e.v.	Myalgia, myasthenia, focal myositis, atrophy of musculature of upper and lower extremities	Herdmyositis

precipitates. The sharply defined fluorescence of the connective-tissue components of the musculature was not evaluated (Figs. 3 and 4).

In the immunofluorescence microscopic examinations, Ag-antibody complexes were detected in the sarcolemma and vessels of /207

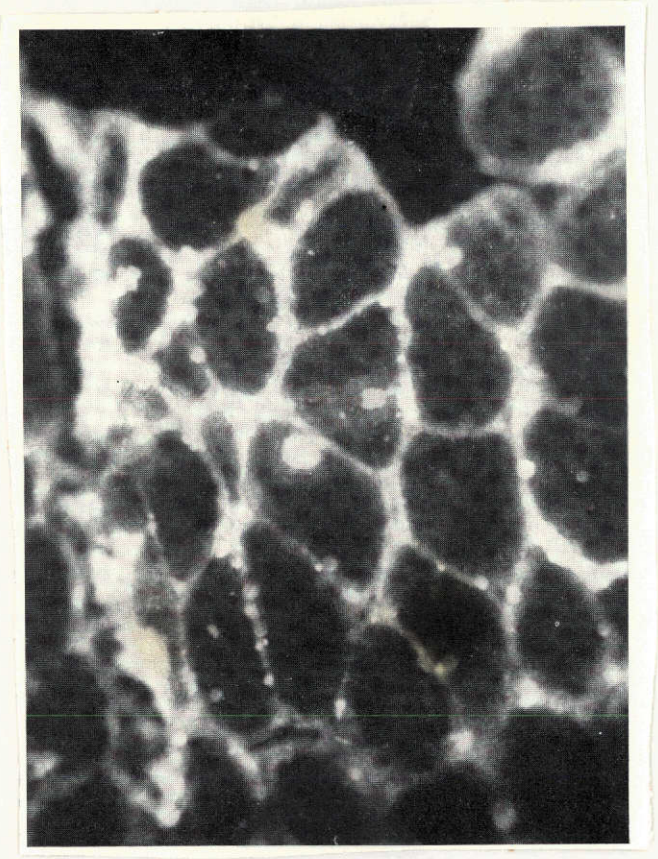

Fig. 3. Sarcolemma fluorescence in severe myositis; direct IF of transversely striated musculature with antihuman IgG/FITC; cross section; at upper edge of picture, capillary cut lengthwise with intense wall fluorescence. The discrete points of fluorescence within the sarcolemmic tubes are orange and produced by Lipofuscin. -- Microscope NF Zeiss, BG 3/2, UG 1/3 -- GG 9/1; Obj. 60 x HI; microprojective K 5:1; exposure time 2 min, magnification 800 x.

five of six patients with L.e.chr.d.l., of which none exhibited myositic symptoms. One of the two patients with L.e.chr.d.d. exhibited immunoglobulin and complement deposition in the sarcolemma without clinical indication. In the six patients studied with L.e.chr.c.ex.s., of which two complained of muscle symptoms, L.e.-typical immunocomplex deposits were detected in varying concentration and localization (Table II).

The most conspicuous pathomorphological changes under the electron microscope are found in the muscle fibers, both in the myofibrils and in the mitochondria (sarcosomes) and the sarcoplasmic reticulum. The myofibrils sometimes exhibit considerably disintegration, the Z-striation being either abnormally widened -- in which case it appears more or less granular -- markedly disoriented, or no longer recognizable as such. Analogously, the myofilaments in such myomeres either follow irregular paths or exhibit granular disintegration.

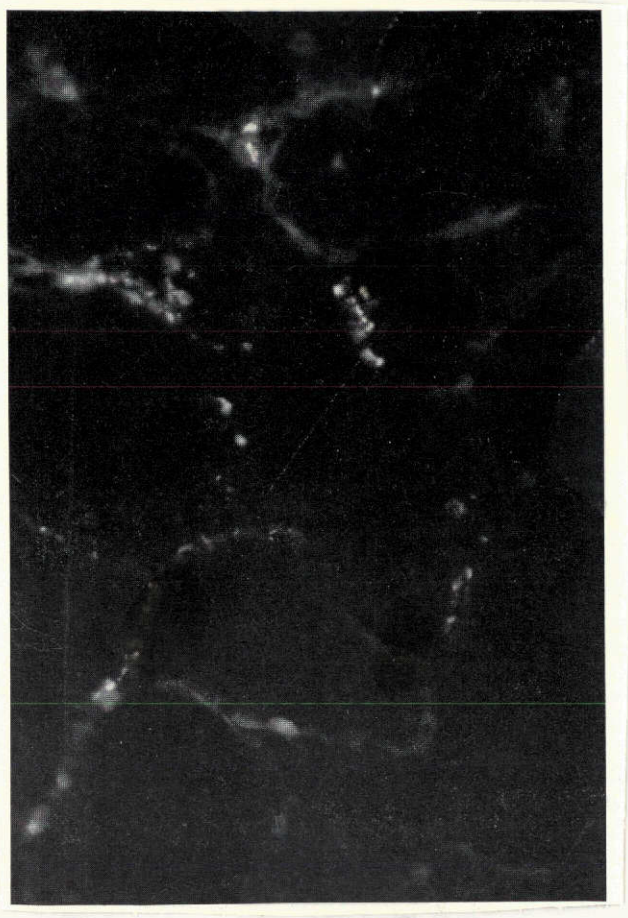

Fig. 4. Fluorescence of capillaries in transversely striated musculature in mild myositis; cross section; direct IF with antihuman β_1C/FITc; technical conditions as in Fig. 3. Exposure time 3 min, magnification 1000 x.

In some cases, there are significant subsarcolemmal increases in mitochondria, the latter exhibiting an abnormal internal structure, while largely or completely disappearing in fibers showing major changes. In most cases, /208 one of the primary features was a subsarcolemmal increase in lipofuscin granula, but only isolated interfibrillar vacuoles could be found, which obviously did not constitute expanded structures of the sarcoplasmic reticulum, which is no longer present in the majority of highly disintegrated fibers. This is also true for glycogen.

In isolated cases, there /209 were filamentous structures bounded by a membrane in the muscle cells. The disintegration and destruction of the parenchyma are more pronounced in visceral than in chronic L.e. There are sometimes a large number of conspicuous folds in the sarcolemma. In the interstitial vessels, the basal membrane in some cases -- primarily in L.e.v. -- is unevenly thickened or splintered, while granular structures and myelin forms in the endothelium were less frequently observed (Fig. 5).

TABLE II. IMMUNOHISTOLOGICAL FINDINGS IN RELATION TO CLINICAL TYPES OF L.e. AND CLINICAL SYMPTOMS OF MUSCLE INVOLVEMENT

Patient	Clinical type of L.e.	Muscle symptoms	Immunhistological findings			
			β_1C	IgA	IgM	IgG
A. E.	L.e.chr.d.l.	ø	S+ C+	ø	C+	C+
R. A.	L.e.chr.d.l.	ø	ø	C+	C+	ø
K. F.	L.e.chr.d.l.	ø	A+ S+	ø	ø	ø
S. R.	L.e.chr.d.l.	ø	ø	ø	ø	ø
K. P.	L.e.chr.d.l.	ø	ø	ø	C(+)	ø
Sch. H.	L.e.chr.d.l.	ø	S+ A+	ø	A+	ø
H. K.	L.e.chr.d.d.	Myalgia of upper extremities	ø	ø	ø	ø
N. P.	L.e.chr.d.d.	ø	S+	S+	S+ C+	ø
V. L.	L.e.chr.c.ex.s.	ø	S+	ø	C(+)	C(+)
K. M.	L.e.chr.c.ex.s.	Myalgia of upper extremities	S+	ø	C(+)	ø
F. C.	L.e.chr.c.ex.s.	ø	ø	ø	C+	ø
M. H.	L.e.chr.c.ex.s.	ø	S+ C+	ø	+	(+)
Z. K. H.	L.e.chr.c.ex.s.	ø	C+	ø	C+	C+

A = Arteriolen mittleren Grades, C = Kapillaren, S = Sarkolemm

A = medium-sized arterioles, C = capillaries,
S = sarcolemma

TABLE III. ELECTRON MICROSCOPIC FINDINGS IN RELATION TO CLINICAL TYPES OF L.e. AND CLINICAL SYMPTOMS OF MUSCLE INVOLVEMENT

Patient	Clinical type of L.e.	Muscle symptoms	Electron microscopic finding
B. U.	L.e.chr.d.l.	ø	+
M. U.	L.e.chr.d.l.	ø	+
A. E.	L.e.chr.d.l.	ø	(+)
Z. A.	L.e.chr.d.l.	ø	+
S. M.	L.e.chr.d.l.	ø	(+)
Sch. M.	L.e.chr.d.l.	ø	+
N. P.	L.e.chr.d.d.	ø	+
Sp. J.	L.e.chr.c.ex.s.	ø	+
V. L.	L.e.chr.c.ex.s.	ø	(+)
K. M.	L.e.chr.c.ex.s.	Myalgia of upper extremities	+
B. E.	L.e.chr.c.ex.s.	ø	(+)
Z. A.	L.e.v.	Myalgia, myasthenia, pronounced atrophy of upper and lower extremities	-
J. W.	L.e.v.		-
H. G.	L.e.v.	Myasthenia of lower extremities	(+)

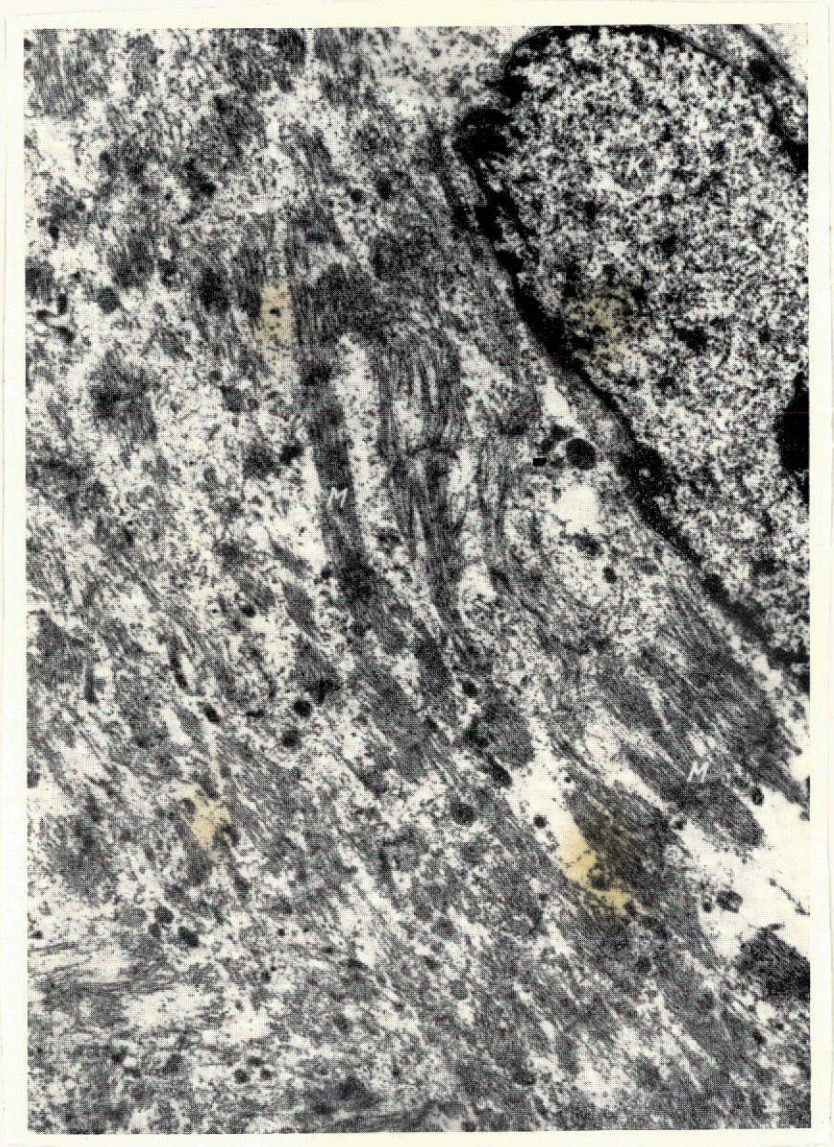

Fig. 5. Part of a muscle fiber in L.e.v. with cell nucleus (K) and largely destroyed myofibrils (M) as well as fragmented myofilaments. Sarcosomes can no longer be clearly recognized. Magnification 20,000 x.

In some cases of L.e.chr., the submicroscopic changes are very indistinct and cannot always be differentiated with certainty, so that it may not be possible to interpret every slight alteration in the sense of a myopathy. We have already discussed elsewhere [7] the sometimes considerable destruction in the paren- /210 chyma, primarily affecting the myofibrils and mitochondria; they

are largely consistent with the histological findings and appear as a whole more massively in L.e.v. than in L.e.chr.

In six patients with L.e.chr.d.l., of which one complained of myositic symptoms, one patient with L.e.chr.d.d. without clinical evidence of muscle involvement, four patients with L.e.chr.c.ex.s., of which one patient exhibited myositic symptoms, and three patients with L.e.v., of which two exhibited myalgias, changes could be detected which were compatible with involvement of muscle in L.e. (Table III).

In the muscle AGCT, only macroscopically visible, marked agglutination was evaluated, as known from the Coombs test and the determination of isoagglutinins.

Compared with the titer of the Coombs serum without additives (2048), the addition of NaCl solution without antigen showed the same titer, while addition of NaCl solution with antigen and of control serum with antigen showed a drop of one stage each (1024). This was the initial value of our evaluation:

512 positive = 1 stage drop = negative
256 positive = 2 stage drop = doubtful
128 positive = 3 stage drop = positive, etc.

In 13 patients with L.e.chr.d.l., of which two complained of myalgias, antibodies could be ruled out with the aid of the muscle AGCT. Two patients exhibitied small quantities of antibodies without clinical indications. The two symptom-free patients with L.e.chr.d.d. and four patients with L.e.chr.c.ex.s., of which two complained of myositic symptoms, shows no antibodies in the muscle AGCT, while three patients with L.e.v. exhibiting clinical symptoms of muscle involvement yielded positive results. The separate muscle AGCT studies on a larger group are presented in relation to clinical picture in Table IV.

TABLE IV. RESULTS OF MUSCLE AGCT OF MIESCHER IN RELATION TO CLINICAL FORM OF L.e.

Clinical form	Patients	M-AGCT Results +	ø
1. L.e.chr.d.l.	46	6	40
Clin. +	5	0	5
Clin. ø	41	6	35
2. L.e.chr.d.d.	5	1	4
Clin. +	1	0	1
Clin. ø	4	1	3
3. L.e.chr.c.ex.s.	13	1	12
Clin. +	2	0	2
Clin. ø	11	1	10
4. L.e.v.	4	3	1
Clin. +	3	3	0
Clin. ø	1	0	1

In the detection of sarcolemma antibodies (S-A) by the method of indirect immunofluorescence [17], there were positive fluorescence reactions in the sarcolemma-subsarcolemma and in the intermyofibrillar region. We considered those sera positive which exhibited a marked fluorescence with a titer of 1:8 or more (Fig. 6).

Sarcolemma antibodies were detected in six of 11 patients with L.e.chr.d.l., of which one complained of muscle symptoms. Two patients with L.e.chr.d.d. and six patients with L.e.chr.c.ex.s., /212 of which one and two respectively complained of muscle pain, exhibited no sarcolemma antibodies. Two patients with L.e.v. and pronounced myositic symptoms exhibited sarcolemma antibodies.

The comparison determinations of antinuclear factors by means of indirect immunofluorescence (Weller and Coons) yielded a clear correlation between ANF and S-A.

The determinations of S-A in relation to clinical form of L.e. and the clinical symptoms of lupus myopathy carried out separately on a larger patient pool are presented in Table V.

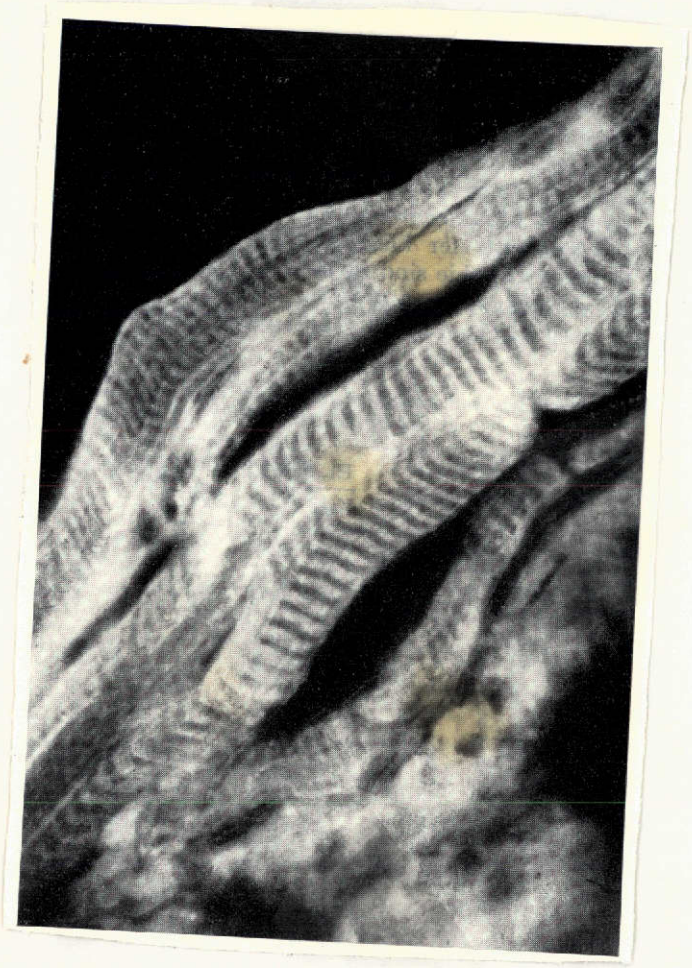

Fig. 6. Antibodies against transversely striated musculature in myasthenia gravis pseudoparalytica, indirect IF, substrate: transversely striated musculature of rabbits; serum dilution 1:50; antihuman IgG/FITC; technical conditions as in Fig. 3. Exposure time 90 sec, magnification 600 x.

The controls in the methods cited were studies conducted for differential diagnosis in other dermatoses.

Discussion

The findings obtained with our patient pool show that the histomorphological studies are the most reliable methods for confirming clinically silent involvement of muscle in L.e. (Table VI). Fluorescence microscopy appear to furnish the most reliable evidence before the light microscopic and electron microscopic examination. It permits early detection of immunological processes, the nature of which as an immune complex can be derived from the localization, the morphological pattern, and the immunoglobulin class. Changes detectable in light microscopy do not permit any conclusions on the etiopathogenesis. The alteration of vascular connective tissue assessed as mesenchymal activation, produced by circulatory disorders of the perimysium in the sense of peristasis, can, like the clinically manifested focal myositis detectable in L.e.v., be of autoimmunological, allergic, or toxic origin.

TABLE V. RESULTS OF SARCOLEMMA-ANTIBODY FLUORESCENCE TEST IN RELATION TO CLINICAL FORM OF L.e.

Clinical form	Patients	S-A-F test +	S-A-F test ø
1. L.e.chr.d.l.	88	23	65
Clin. +	14	3	11
Clin. ø	74	20	54
2. L.e.chr.d.d.	5	0	5
Clin. +	1	0	1
Clin. ø	4	0	4
3. L.e.chr.c.ex.s.	12	4	8
Clin. +	2	0	2
Clin. ø	10	4	6
4. L.e.v.	8	5	3
Clin. +	3	3	0
Clin. ø	5	2	3

TABLE VI. COMPARISON OF RESULTS OF VARIOUS PARACLINICAL METHODS FOR DETECTING MUSCLE INVOLVEMENT IN L.e. IN RELATION TO FORM AND MYOPATHIC SYMPTOMS

Clinical form	Histo			IF			El.Micr.			M-AGCT			S-A			ANF		
	No.	+	ø	No.	+	ø	No.	+	ø	No.	+	ø	No.	+	ø	No.	+	ø
L.e.chr.d.l.	19	8	11	6	4	2	10	10	0	15	2	13	16	7	9	22	4	18
Clin. +	2	0	2	0	0	0	1	1	0	2	0	2	1	1	0	2	1	1
Clin. ø	17	8	9	6	4	2	9	9	0	13	2	11	15	6	9	20	3	17
L.e.chr.d.d.	3	2	1	2	1	1	1	1	0	2	0	2	3	0	3	3	0	3
Clin. +	1	1	0	1	0	1	0	0	0	0	0	0	1	0	1	1	0	1
Clin ø	2	1	1	1	1	0	1	1	0	2	0	2	2	0	2	2	0	2
L.e.chr.c.ex.s.	7	4	3	5	5	0	4	4	0	5	0	5	6	2	4	8	0	8
Clin. +	1	1	0	2	2	0	1	1	0	2	0	2	2	0	2	2	0	2
Clin. ø	6	3	3	3	3	0	3	3	0	3	0	3	4	2	2	6	0	6
L.e.v.	2	2	0	0	0	0	3	3	0	3	3	0	4	4	0	4	3	1
Clin. +	2	2	0	0	0	0	1	1	0	3	3	0	3	3	0	3	2	1
Clin. ø	0	0	0	0	0	0	2	2	0	0	0	0	1	1	0	1	1	0

The electron microscope findings represent conditions resulting from the alteration of the myofibrils and the sarcoplasmic reticulum, conditions which can have various causes.

The results of the muscle AGCT to detect humoral muscle antibodies indicate that, following primary vascular processes,

which can be detected very early by direct immunofluorescence, nonspecific inflammatory and degenerative changes take place in the muscle, which are detectable under the light and electron microscopes, and which finally produce antibodies against the musculature. /213 They constitute the so-called secondary phenomenon and are evidence of antibodies. Because of their late appearance, they are well suited for indicating a late stage in involvement of muscle in L.e., the clinically silent early stage of which can be detected by histomorphological methods.

No relationship was found between the muscle AGCT on one hand and the histomorphological results and clinical symptoms on the other. Neither was there a correlation to the results of the method of indirect immunofluorescence.

Obviously, the method of indirect immunofluorescence to determine sarcolemma antibodies does not detect specific antibodies, since the results appear independent of clinical symptoms, histomorphological changes, and muscle AGCT. However, there is a clear correlation between sarcolemma antibodies and ANF, a discovery which could point to similar antigenic determinants.

Moreover, it is advisable to select a limit titer, not obtained by examination of normal persons, but instead from a group of L.e. patients without lupus myopathy, since the nonspecific humoral activity in L.e. is obviously higher and can simulate a specific antibody.

The changes in the form of mesenchymal activation detected under the light microscopic in large numbers in L.e.chr.d.l. must be considered a manifestation of the general immunological processes observed in the musculature without claim to specificity, given the ambiguous electron-microscopic and immunohistologically negative findings, the usually negative result of the muscle AGCT,

and the frequent detection of sarcolemma antibodies in the absence of clinical symptoms.

The changes in the sense of a focal myositis [9, 10, 14, 16] detected under the light microscope in L.e.chr.d.d. are confirmed by the immunofluorescence microscopic detection of immune complexes. The absence of humoral antibodies in the muscle AGCT points to low-order immunological activity and, together with the morphological findings, characterizes the L.e.chr.d.d. in the absence of clinical symptoms of a lupus myopathy as a chronic phase of a viscerally manifested form of L.e. Histomorphologically and in relation to humoral antibodies, L.e.chr.c.ex.s. behaves like a classical chronic L.e. This underscores its status as a chronic form of L.e. and suggests that its acute features are restricted to the skin symptoms.

In L.e.v. with pronounced symptoms, the histological and immunohistological changes characteristic of lupus myopathy must be anticipated. The humoral antibodies which are always detected in the muscle AGCT and the sarcolemma-antibody test in L.e.v., regardless of clinical signs of involvement of muscle and morphological changes in the sense of focal myositis, characterize the degree of immunological activity in L.e.v. and suggest the subordinate pathogenetic significance of humoral antibodies in organic lesions. They can be viewed as immunological side effects, the major significance of which is as a parameter of the immune mechanisms.

One result of the present investigations is that the histomorphological methods, primarily the immunofluorescence microscopic studies, appear well suited for detecting clinically silent involvement of muscle in L.e. The humoral antibodies detectable with the muscle AGCT are evidence of antibodies which does not appear suitable for early detection of clinically silent lupus

myopathy, but which does acquire significance in the determination of the clinical stage of a L.e. myositis. The specificity of the /214
sarcolemma antibody must be considered dubious. A more precise statement on its diagnostic significance can be anticipated after determination of a more suitable limit titer.

Based on separate light microscopic determination, the changes in the sense of mesenchymal activation observed in L.e.chr.d.l. are considered nonspecific in the sense of a partial symptom of a general immunological process manifesting itself in the musculature. The findings obtained in L.e.chr.c.ex.s. support the view that the acute symptoms in this clinical form of L.e. are restricted to the skin. On the other hand, L.e.chr.d.d. exhibits histomorphological and humoral findings corresponding to those of L.e.v. and suggest that L.e.chr.d.d. could be a chronic variant of L.e.v.

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